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NEW OR UNUSUAL RECORDS

New records of *Cylindrocladium* and *Cylindrocladiella* spp. in South Africa

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Three species of *Cylindrocladium* (*Cm.*) and three of *Cylindrocladiella* (*Ca.*) were collected from forestry plantations and nurseries in South Africa. These fungi include species previously recorded in South Africa, *Cm. scoparium* and *Ca. parva*, as well as new records for the country: *Cm. clavatum*, *Cm. colhounii*, *Ca. camelliae* and an undescribed *Cylindrocladiella*. Single-conidium isolates of all six fungi tested were pathogenic to *Eucalyptus grandis*, *Medicago truncatula*, *Arachis hypogaea*, *Glycine max* and *Pisum sativum*. Only *Cm. clavatum* was pathogenic to *Solanum tuberosum*. *Cm.* species were more virulent than the *Ca.* species, with *Cm. clavatum* being the most virulent of all the fungi.

INTRODUCTION

Information concerning the occurrence and importance of *Cylindrocladium* Morgan (*Cm.*) and *Cylindrocladiella* Boesewinkel (*Ca.*) spp. in South Africa is limited. There are several reports of *Cm. scoparium* Morgan (Darvas *et al.*, 1978; Lamprecht, 1986; Hagemann & Rose, 1988; Crous *et al.*, 1991) and *Ca. parva* (Anderson) Boesewinkel (Darvas *et al.*, 1978; Crous *et al.*, 1991). However, pathogenicity of *Cm. scoparium* has been established only on *Medicago truncatula* cv. Borung, *Pinus* spp. and *Acacia longifolia* (Darvas *et al.*, 1978; Hagemann & Rose, 1988; Lamprecht, 1986). Pathogenicity of *Ca. parva* has been demonstrated on *Pinus* spp. (Darvas *et al.*, 1978).

During routine investigations of *Eucalyptus* nurseries and plantations a number of *Cylindrocladium* and *Cylindrocladiella* spp. were isolated. The aim of this study was to identify and report the presence of these fungi in South Africa. In an attempt to determine their relative importance, pathogenicity was determined on *Eucalyptus* and several other important crops growing in the regions where the isolates were collected.

MATERIALS AND METHODS

Diseased seedlings and *Eucalyptus* clonal cuttings (roots, shoots and leaves) were surface-disinfested for 1 min in 1% NaOCl (prepared by diluting a commercial sodium hypochlorite bleach), rinsed with sterile water, and placed in moist chambers for 3 days at 25°C with 12 h light/day. Samples of seedling potting medium, as well as soil samples taken to a depth of 15 cm from within *Eucalyptus* clonal orchards or plantations, were placed in Petri dishes and moistened. Azalea leaves were inserted half-way into the soil or potting medium (Linderman, 1972; 1974), and the plates were sealed. Azalea leaves were examined 14 days later and single-conidium isolates were made on 2% malt extract agar (MEA) (20 g Oxoid malt extract, 15 g Difco agar, 1000 ml H_2O), and maintained on MEA slants at 25°C. Single-conidium isolates were grown on carnation-leaf agar (CLA) (Crous *et al.*, 1992) at 25°C for 7 days under cool-white fluorescent illumination, and their morphological characteristics were determined. Representative cultures were lodged with the National Collection of Fungi, Pretoria (PPRI).

Three *Cylindrocladium* and three *Cylindrocladiella* spp. were detected, namely *Cm. clavatum* Hodges & May (PPRI 3996), *Cm. scoparium* (PPRI 3909), *Cm. colhounii* Peerally (PPRI 4000), *Ca. camelliae* (Venkatar *et* Venkata Ram) Boesewinkel (PPRI 3990), *Ca. parva* (PPRI 3999) and an undescribed *Ca.* species (PPRI 4050). Identifications were confirmed by comparing isolates with type cultures and specimens. These were the following: *Cm. clavatum* (BPI 414550), *Cm. sco-*

Table 1. A comparison of morphological features of *Cylindrocladium* (*Cm.*) and *Cylindrocladiella* (*Ca.*) spp. occurring in South Africa

Species	Accession number	Vesicle shape	Conidium		Ascospore	
			Septation	Dimensions (μm)[a]	Septation	Dimensions (μm)[a]
Cm. clavatum	PPRI 3996	Clavate	1	44·0 × 4·5	—	—
Cm. scoparium	PPRI 3909	Ellipsoid to obpyriform	1	45·0 × 4·0	—	—
Cm. colhounii	PPRI 4000	Clavate	1-(3)	90·0 × 6·5	3	51·0 × 6·0
Ca. camelliae	PPRI 3990	Ellipsoid to lanceolate	1	12·0 × 2·0	—	—
Ca. parva	PPRI 3999	Pyriform	1	17·0 × 2·0	—	—
Ca. species	PPRI 4050	Ellipsoid to clavate	1	15·5 × 2·0	—	—

[a] Measurements represent averages of 50 observations on carnation-leaf agar.

parium (type on *Gleditsia triacanthos* L., BPI), *Cm. colhounii* (IMI 167581), *Ca. camelliae* (IMI 47717) and *Ca. parva* (ATCC 28272). The new *Ca.* species did not match the description of any other species in the genus (Boesewinkel, 1982). *Cylindrocladium* and *Cylindrocladiella* spp. occurring in South Africa are compared morphologically in Table 1.

Cm. scoparium was found on *Eucalyptus*, *Pinus* and *Acacia* spp. in the Cape, Transvaal and Natal Provinces. *Cm. colhounii* was found only on eucalypts in the Eastern Transvaal, while *Cm. clavatum* was isolated only from eucalypts in Natal. *Ca. camelliae* was isolated from eucalypts in the Transvaal and Natal Provinces. The undescribed *Ca.* species was found on forest litter throughout the Transvaal and isolated from the roots of peanuts intercropped with *Eucalyptus* in Natal. *Ca. parva*, which had previously been found on *Pinus* and *Protea* roots in Natal and Transvaal (Crous *et al.*, 1991), was isolated from *Pinus* roots in the Western Cape in this study.

Pathogenicity was tested on *Eucalyptus grandis*. In addition the isolates were also tested for their ability to cause disease on other crops commonly cultivated in the regions from which the isolates were collected. These were *Medicago truncatula* cv. Borung (alfalfa), *Glycine max* cv. Ibis (soybean), *Arachis hypogaea* cv. Sellie (peanut), *Pisum sativum* cv. Novella (pea) and *Solanum tuberosum* cv. Vanderplank (potato).

One isolate of each *Cylindrocladium* and *Cylindrocladiella* sp. was subcultured on MEA and incubated for 14 days at 25°C under 12 h light/day. Erlenmeyer flasks (500 ml) were filled with 300 g of a soil:bran:H_2O mixture (10:1:4 w/w), and autoclaved for 15 min at 103 kPa on three consecutive days. Agar discs from leading edges of the various fungal colonies were added to the cooled medium and incubated for 14 days as described above. Flasks were shaken by hand every second day to ensure thorough colonization of the medium and to prevent the inoculum from forming lumps. Inoculum of each fungus was mixed with vermiculite in the ratio of 1:1 (w:w). The infested medium was mixed thoroughly and dispensed into 500-ml plastic pots. Two-month-old plants grown in a steam-sterilized sandy loam were carefully uprooted and repotted individually in the inoculum/vermiculite mixture. Potato tubers were wounded by removing a 3-mm-diameter plug (2 cm deep). They were then buried 5 cm deep in 500-ml pots filled with inoculum/vermiculite mixtures of the respective fungi. Control tubers were buried in a sterile mixture.

Experiments were conducted in a glasshouse covered with shade cloth, kept at 27°C (day) and 22°C (night). Pots were watered daily, incorporating a soluble fertilizer (Wonder 3:2:1 (22) Supranure Plus at the rate of 10 ml/l H_2O) every 7 days. There were ten replicates of each host for each fungal treatment. Controls consisted of ten plants of each host transplanted to sterile vermiculite mixture. All inoculation experiments were repeated using freshly grown plants.

Disease was assessed after 30 days, and average disease score rated on a scale of 0–4, where 0 = roots healthy; 1 = root discoloration; 2 = root and stem discoloration; 3 = plants wilted or tubers rotted; 4 = plants dead. To complete Koch's postulates, re-isolations were made from the diseased plants as well as from the margins of the rotted areas on the potatoes. Diseased tissue was surface sterilized for 1 min in 1% NaOCl and

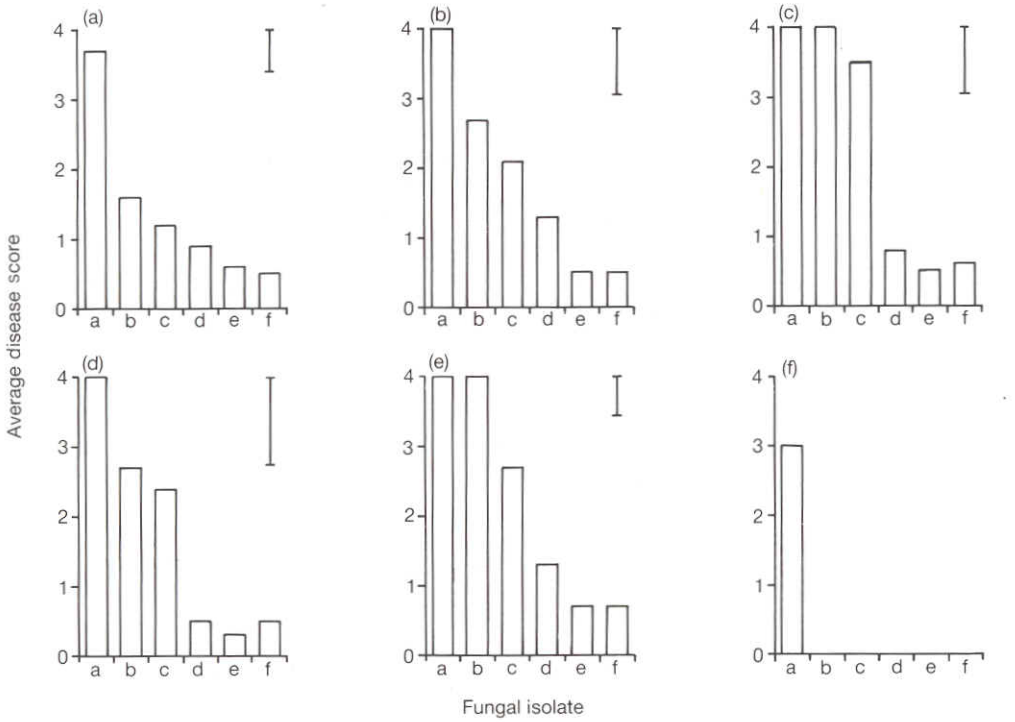

Fig. 1. Average disease score caused by *Cylindrocladium* and *Cylindrocladiella* spp. to various hosts in glasshouse trials. Scale bar = LSD ($P = 0{\cdot}01$). Hosts: (a) peanuts; (b) peas; (c) alfalfa; (d) soybean; (e) *Eucalyptus*; (f) potato. Fungi: a, *Cm. clavatum*; b, *Cm. scoparium*; c, *Cm. colhounii*; d, *Ca. camelliae*; e, *Ca. parva*; f, *Ca. species*.

plated on potato-dextrose agar (PDA) (Biolab) supplemented with 50 mg/l chloramphenicol and 50 mg/l streptomycin sulphate.

RESULTS AND DISCUSSION

The average disease score for each species did not differ significantly between the two experiments. The results were pooled and are represented in Fig. 1. All *Cylindrocladium* and *Cylindrocladiella* spp. were pathogenic to the hosts tested, except potatoes, to which only *Cm. clavatum* was pathogenic (Fig. 1(f)). *Cylindrocladium* spp. were more virulent than *Cylindrocladiella* spp. while *Cm. clavatum* was the most virulent on all hosts, followed by *Cm. scoparium* and *Cm. colhounii*. The various symptoms in order of severity included damping-off of alfalfa, seedling blight of eucalypts, yellowing, wilt and death of soybean, peas and peanuts, and tuber rot of potatoes.

Among the three *Cylindrocladiella* spp. tested, the average disease score of *Ca. camelliae* on *Eucalyptus* was significantly ($P = 0{\cdot}01$) more than that of the other two *Cylindrocladiella* spp. On the other five hosts, no significant differences in virulence between the three *Cylindrocladiella* spp. could be detected. *Cylindrocladium* spp. were re-isolated from the stems and roots of all inoculated plants, whereas *Cylindrocladiella* spp. were re-isolated only from the finer roots. *Ca. camelliae*, however, was also re-isolated from *Eucalyptus* stems.

In addition to tuber rot, *Cm. clavatum* also induced superficial, circular necrotic spots on tubers, with the underlying areas developing a dry, corky rot up to 3 mm deep. These symptoms are similar to those observed on potato in Brazil (Bolkan *et al.*, 1980, 1981). The wide host range and high level of virulence of *Cm. clavatum* indicate that it could be an important root pathogen of agronomic and forest crops in South Africa. This fungus was re-isolated from completely decayed tubers, from superficial tuber lesions, and from roots and stems.

Although *Cylindrocladium* and *Cylindrocladiella* spp. have been recorded only rarely in South Africa, this study has shown that these fungi are widely distributed in forest regions of

the country. *Cm. clavatum* is known to be an important pathogen of eucalypts, peanut, soybean, potato, peas and alfalfa elsewhere in the world (Bolkan *et al.*, 1981: Lopes & Reifschneider, 1982: Ooka & Uchida, 1983; Dianese *et al.*, 1986) but was found only on *Eucalyptus* spp. in this study.

With the exception of peas, *Cm. scoparium* had previously been isolated from all the other hosts tested in this study (Sobers & Littrell, 1974; Almeida & Bolkan, 1981; Ooka & Uchida, 1983; Barnard, 1984; Lamprecht, 1986). *Cm. colhounii*, *Ca. camelliae* and *Ca. parva* were all shown to infect peanuts, peas, alfalfa, soybean and *Eucalyptus* in this study. Previously, these three pathogens had been reported on *Eucalyptus* (Sharma & Mohanan, 1982; Mohanan & Sharma, 1985; Nair & Jayasree, 1987), but apart from a report of *Cm. colhounii* on peanuts (Rossman, 1983) they had not been recorded on peas, alfalfa, peanuts or soybean. Furthermore, the undescribed *Ca.* species was isolated from peanut roots as well as *Eucalyptus* leaf litter in this study. Our results indicate that most of these fungi can be pathogenic to a wider range of hosts than was previously realized.

Soybeans and peanuts are frequently used as cover crops in agroforestry to control weed growth and to improve the nitrogen status of soils in young eucalypt plantations. It is thus possible that infestation of soil and severe infection of the cover crops might occur through planting of diseased or contaminated eucalypt cuttings or seedlings.

REFERENCES

Almeida OC de, Bolkan HA, 1981. Pathogenicity of four *Cylindrocladium* species to groundnut, soybean, *Eucalyptus* and potato tubers. *Fitopatologia Brasileira* **6,** 237–44.

Barnard EL, 1984. Occurrence, impact, and fungicidal control of girdling stem cankers caused by *Cylindrocladium scoparium* on *Eucalyptus* seedlings in a south Florida nursery. *Plant Disease* **68,** 471–3.

Boesewinkel HJ, 1982. *Cylindrocladiella*, a new genus to accommodate *Cylindrocladium parvum* and other small-spored species of *Cylindrocladium*. *Canadian Journal of Botany* **60,** 2288–94.

Bolkan HA, Dianese JC, Ribeiro WRC, De Almeida OC, 1980. Disease caused by *Cylindrocladium* on potato tubers in Brazil. (Abstract) *Plant Disease* **64,** 225.

Bolkan HA, Ribeiro WRC, De Almeida OC, 1981. Pathogenicity of *Cylindrocladium clavatum* causing potato tuber rot. *Plant Disease* **65,** 47–9.

Crous PW, Phillips AJL, Wingfield MJ, 1991. The genera *Cylindrocladium* and *Cylindrocladiella* in South Africa, with special reference to forestry nurseries. *South African Forestry Journal* **157,** 69–85.

Crous PW, Phillips AJL, Wingfield MJ, 1992. Effects of cultural conditions on vesicle and conidium morphology in species of *Cylindrocladium* and *Cylindrocladiella*. *Mycologia* **84,** 497–504.

Darvas JM, Scott DB, Kotzé JM, 1978. Fungi associated with damping-off in coniferous seedlings in South African nurseries. *South African Forestry Journal* **104,** 15–19.

Dianese JC, Ribeiro WRC, Urben AF, 1986. Root rot of soybean caused by *Cylindrocladium clavatum* in central Brazil. *Plant Disease* **70,** 977–80.

Hagemann GD, Rose PD, 1988. Leaf spot and blight on *Acacia longifolia* caused by *Cylindrocladium scoparium*: a new host record. *Phytophylactica* **20,** 311–16.

Lamprecht SC, 1986. A new disease of *Medicago truncatula* caused by *Cylindrocladium scoparium*. *Phytophylactica* **18,** 111–14.

Linderman RG, 1972. Isolation of *Cylindrocladium* from soil or infected azalea stems with azalea leaf traps. *Phytopathology* **62,** 736–9.

Linderman RG, 1974. The role of abscised *Cylindrocladium* infected azalea leaves in the epidemiology of *Cylindrocladium* wilt of azalea. *Phytopathology* **63,** 553–8.

Lopes CA, Reifschneider FJB, 1982. Pathogenicity of *Cylindrocladium clavatum* to pea, a new host, and preliminary evaluation of its virulence. *Plant Disease* **66,** 951–3.

Mohanan C, Sharma JK, 1985. *Cylindrocladium* causing seedling diseases of *Eucalyptus* in Kerala, India. *Transactions of the British Mycological Society* **84,** 538–9.

Nair JM, Jayasree MC, 1987. Occurrence of *Cylindrocladium colhounii* Peerally on eucalypts in Kerala. *Current Science* **55,** 799–800.

Ooka JJ, Uchida JY, 1983. *Cylindrocladium* root and crown rot of alfalfa in Hawaii. *Plant Disease* **66,** 947–8.

Rossman AY, 1983. The phragmosporous species of *Nectria* and related genera. *Mycological Papers* **150,** 1–164.

Sharma JK, Mohanan C, 1982. *Cylindrocladium* spp. associated with various diseases of *Eucalyptus* in Kerala. *European Journal of Forest Pathology* **12,** 129–36.

Sobers EK, Littrell RH, 1974. Pathogenicity of three species of *Cylindrocladium* to select hosts. *Plant Disease Reporter* **58,** 1017–19.